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14. ABSTRACT

There has been a major focus on the androgen receptor (AR) pathway as the principal therapeutic target for CRPC including recently approved therapies such as next-generation antiandrogen enzalutamide and abiraterone. Despite these advances that provide temporary respite, almost all patients will go on to die from progressive and resistant prostate cancer. Therefore, there is an urgent need to identify resistant pathways that perpetuate disease progression. We provided preliminary data demonstrating that p52 increases AR variant V7 (AR-V7) expression and enhances prostate cancer cell resistance to next-generation antiandrogen enzalutamide treatment. We hypothesize that overexpression of p52 signaling activates resistance pathways to enzalutamide and co-targeting p52 will overcome treatment resistance. In this project, we will examine the potential mechanisms underlying p52-mediated treatment resistance (Aim 1). Aim 2 will validate the efficacy of co-targeting p52 to overcome treatment resistance to enzalutamide. We hope to identify the mechanisms of adaptive/resistant pathways that are responsible for enzalutamide resistance, and provide a rationale for therapeutic co-targeting to overcome enzalutamide resistance.

15. SUBJECT TERMS
NF-κB2/p52, Androgen receptor, Variants, enzalutamide, resistance

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Introduction

Background: Accumulating evidence suggests that abnormal activation of androgen receptor (AR) including AR variants such as AR-V7 contributes to castration-resistant prostate cancer (CRPC) growth. There has been a major focus on the androgen receptor (AR) pathway as the principal therapeutic target for CRPC including recently approved therapies such as next-generation antiandrogen enzalutamide and abiraterone. Despite these advances that provide temporary respite, almost all patients will go on to die from progressive and resistant prostate cancer. Therefore, there is an urgent need to identify resistant pathways that perpetuate disease progression during an effective AR blockade. NF-κB functions as a master transcription factor in regulating the expression of genes implicated in cell survival and chemo resistance. Numerous studies demonstrate that non-canonical NF-κB2/p52 (p52) is overexpressed in prostate cancer and overexpression of p52 facilitates CRPC progression through activating AR signaling and protecting cells from apoptotic death. We provided preliminary data demonstrating that p52 increases AR variant V7 (AR-V7) expression and enhances prostate cancer cell resistance to next-generation antiandrogen enzalutamide treatment.
Hypothesis: We hypothesize that overexpression of p52 signaling activates resistance pathways to enzalutamide and co-targeting p52 will overcome treatment resistance.
Specific aims: 1. Determine the potential mechanisms of p52-mediated treatment resistance in prostate cancer cells. 2. Co-targeting p52 to overcome treatment resistance to enzalutamide.

Keywords
NF-κB2/p52, Androgen receptor, Variants, enzalutamide, resistance

Accomplishments

We have made significant progress in Major Task 2 and 3: Determining the potential mechanisms underlying p52-mediated AR-V7 production. We demonstrated the Let-7c:Lin28 axis may regulate the resistance of prostate cancer cells expressing p52 to enzalutamide by modulating the expression of AR and its variants.

We reported earlier that activation of p52 promotes progression to CRPC and enzalutamide resistance via the generation of AR variants, specifically AR-V7. Since splice variants such as AR-V7 have been implicated in the resistance of prostate cancer cells to androgen-axis targeting therapeutics such as enzalutamide or abiraterone, we analyzed the effects of Let-7c on the sensitivity of LNCaP cells to enzalutamide or abiraterone. We transfected antisense Let-7c into LNCaP cells and measured the survival of LNCaP cells when treated with 20 μM each of enzalutamide or abiraterone. The results showed that downregulation of Let-7c abolished the sensitivity of LNCaP cells to either enzalutamide or abiraterone (Fig. 1A), indicating that Let-7c may play a major role in the sensitivity of prostate cancer cells to AR-targeted therapeutics. Similarly, overexpression of Let-7c resensitized p52 overexpressing LN-p52 cells to enzalutamide and abiraterone (Fig. 1B). We confirmed these results by transfecting Let-7c into C4-2B-Enza-R and 22Rv1-Enza-R cells generated by chronic culture in enzalutamide-containing

media and which exhibit resistance to enzalutamide. Overexpression of Let-7c resensitized both cell lines to enzalutamide (Fig. 1C & D), demonstrating that suppression of Let-7c expression may be one of the mechanisms mediating the acquisition of resistance to enzalutamide.

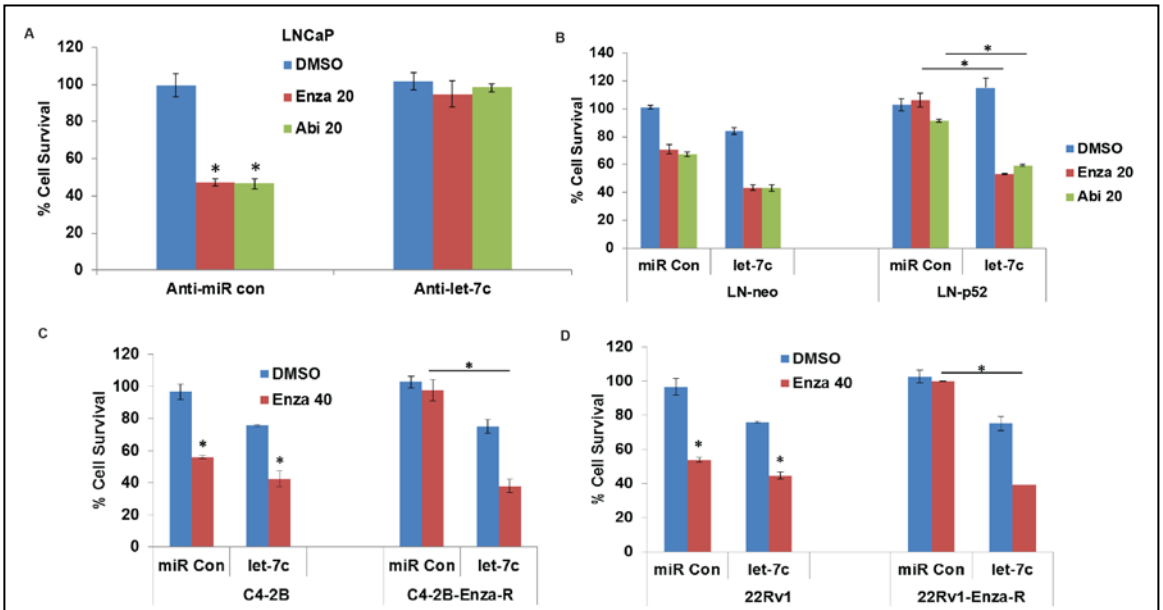

Fig 1. Let-7c involved in resistance to enzalutamide and abiraterone

Using qPCR, we showed that LNCaP cells transiently transfected with p52 and LN-p52 cells express lower levels of Let-7c and higher levels of Lin28, compared with the control LN-neo cells (Fig. 2A & 2B), indicating that p52 may regulate the expression of Let-7c and Lin28 in a reciprocal manner. We confirmed these results using LNCaP cells expressing tet-inducible p52. Induction of p52 expression using Dox reduced the levels of Let-7c, while upregulating Lin28 (Fig. 2C). We also found that in addition to higher expression of Lin28, LN-p52 cells also express higher levels of c-Myc (Fig. 2D & E). Downregulation of either Lin28 or c-Myc using shRNA enhanced the expression of

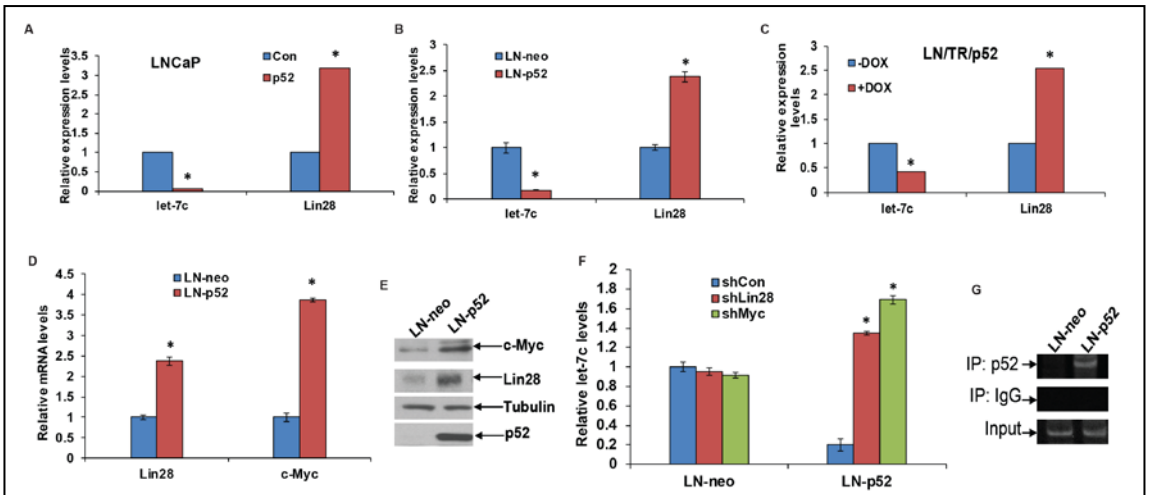

Fig 2. P52 enhances Lin28 expression and inhibits Let-7c expression

Let-7c in LN-p52 cells (Fig. 2F), demonstrating that p52 may control the Let-7c:Lin28:c-Myc axis. ChIP assays revealed that p52 is recruited to and activates the Lin28 promoter (Fig. 2G). These results collectively confirm that p52 modulates Let-7c via Lin28 and c-Myc.

We analysed whether downregulation of Lin28 can affect the resistance of LN-p52 cells to enzalutamide. The results showed that the suppression of Lin28 expression resensitized LN-p52 cells to enzalutamide (Fig. 3A). Analysis of the mechanisms revealed that suppression of Lin28 reduced the expression levels of both FL AR and AR-V7 in LN-p52 cells (Fig. 3B). Taken together, these results established that the Let-7c:Lin28 axis may regulate the resistance of prostate cancer cells expressing p52 to enzalutamide by modulating the expression of AR and its variants.

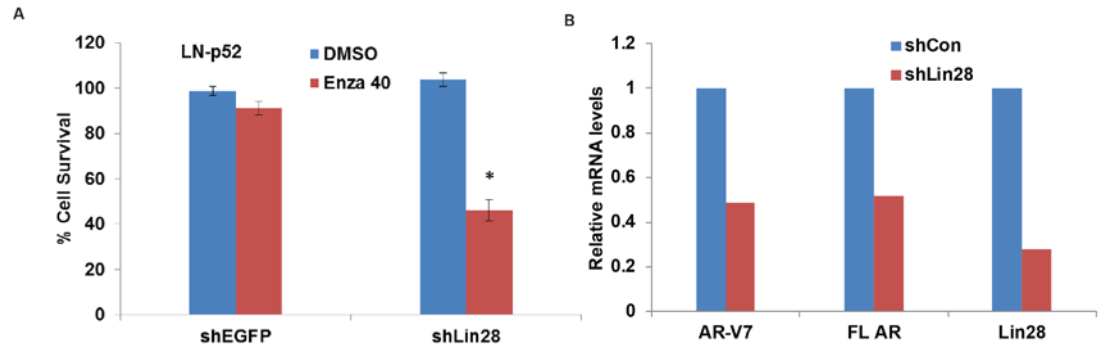

Fig 3. Lin28 mediates p52 induced resistance to enzalutamide

We plan to target p52 in combination with enzalutamide in enzalutamide resistant C4-2BMDVR cells in vitro and in vivo. We will design specific siRNAs targeting p52 and generate bioengineered p52 siRNAs for in vitro and in vivo testing.

Key outcomes:

- We demonstrated that p52 enhances enzalutamide resistance.
- We demonstrated that p52 induces ARv7 expression.
- We demonstrated that p52 induced enzalutamide resistance is mediated by ARv7.
- We demonstrated that p52 regulates AR-V7 expression via Lin28/let7c
- We demonstrated that enzalutamide resistant cells express higher levels of p52, ARv7, Lin28.

Impact

This proposes studies will not only uncover a novel pathway involved in resistant CRPC development, but may also provide proof-of-concept experiments for future development of therapies targeting resistant pathways that are responsible for acquired treatment resistance, and to increase the magnitude and duration of the benefits of second-generation antiandrogen.

Changes/problems
N/A

Products

Publications:

1. Tummala R, Nadiminty N., Lou W, Evans CP, Gao AC. Lin28 induces resistance to anti-androgens via promotion of AR splice variant generation. Prostate 76(5):445-55, 2016. PMID:26714839.

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